# Chapter 6. Study of Camptothecin release from CPT/β-CD and CPT/β-CD-g-Alg inclusion complexes

## 6.1 Synopsis

*This chapter details the release profiles of CPT molecules from CPT/β-CD and CPT/β-CD-g-Alg inclusion complexes, specially synthesised and characterised in Chapter 5. Each complex was held within a dialysis membrane for the release experiments. The results were compared with the release of free CPT through the dialysis membrane. Fick's second law equation was used as a model to calculate the diffusion coefficients. It was found that the CPT/β-CD-g-Alg inclusion complex showed a slower release than the CPT/β-CD inclusion complex and also showed no burst release of CPT unlike that observed for the CPT/β-CD inclusion complex. This is an important finding for use of these complexes when prolonged drug release is required.*

## 6.2 Introduction

Na-Alg polysaccharide has received a great deal of attention in the pharmaceutical industry due to its biocompatibility, non-toxicity and bioadhesion. The term bioadhesion has been used to describe the ability of biological macromolecules and hydrocolloids to adhere to biological tissues [201]. There are two different classes of bioadhesive polymer, the anionic polymers such as Na-Alg and the cationic polymers such as chitosan [202]. The term mucoadhesion is used if one of the surfaces is a mucosal layer [203]. In the last decade, bioadhesive polymers have been of significant interest in controlled release systems [201, 202]. The main reasons for use are prolonged residence time at the site of drug absorption and increased contact to the absorbing mucosa. This results in a steep concentration gradient in favour of drug absorption and localisation in specified regions to improve and enhance the overall drugs effectiveness and bioavailability [202].

Mucoadhesive polymers are based on a polymer-mucin interactions through chemical non-covalent bonds such as hydrogen bonds, van der Waals forces and ionic interactions [203]. Many studies have shown that polyanionic polymers are more effective bioadhesives than polycationic polymers or non-ionic polymers due to the numerous hydrogen bonds generated between the hydrophilic functional groups (COOH and OH) and mucosal surfaces [204, 205].

Na-Alg, which has carboxyl end groups (see Chapter 1, Figure 1.1), is classified as an anionic mucoadhesive polymer [203]. Na-Alg mucoadhesion studies have shown that Na-Alg has the highest mucoadhesive strength when compared to polymers such as chitosan, polystyrene and carboxymethylcellulose due to Na-Alg polymers having numerous carboxylic acid and hydroxyl groups [205, 206].

Most of the reported research on Na-Alg has attempted to formulate Na-Alg as a matrix to retard drug release by introducing cyclodextrin (CD) molecules into the NaAlg structure [128, 188, 207]. β-CD, which is well known as a host molecule, is commonly used in pharmaceutical formulations to enhance drug solubility, stability and bioavailability (see Chapter 1, Figure 1.15) [108]. For release studies, in general, the unique properties of β-CD as carriers helps keep drug molecules in solution and

delivers them to the surface of the dialysis membrane [208]. β-CD itself cannot pass through a dialysis membrane due to its high molecular weight (1134.98 g $mol^{-1}$) [208].

Pluemsab et al. [188] prepared α-CD-alginate by chemically modifying the hydroxyl groups of Na-Alg with cyanogen bromide (CNBr). They found that α-CD-alginate showed an ability to form an inclusion complex with *p*–nitrophenol. β-CD grafted alginate synthesised by Zhang et al. [128] showed that an inclusion complex could be formed with the water-soluble drug (neutral red, NR) [128]. They observed that the β-CD-g-Alg exhibited a controlled release of NR, reaching an equilibrium after 32 h [128]

## 6.3 Diffusion coefficient modelling

The release of CPT molecules from CPT/β-CD and CPT/β-CD-g-Alg inclusion complexes can be considered to take place by diffusion [209]. In order to compare the diffusion rates of free CPT and CPT from the CPT/β-CD and CPT/β-CD-g-Alg inclusion complexes Fick's second law was applied to the data obtained from UV-Vis spectrometry measurements, as discussed further in this chapter. Fick's second law is generally used to describe the diffusion controlled release of the solute concentration variation in a flat sample ($C$) as a function of time ($t$) and distance ($x$) (Equation 6.1) [81, 93, 149, 209, 210].

$$\frac{\partial C}{\partial t} = D\,\frac{\partial^2 C}{\partial \mathcal{X}^2}$$

Equation 6.1

Where the diffusion coefficient $D$ is assumed to be constant and the boundary conditions are:

$$t = 0, \quad -\frac{1}{2}\,l < \mathcal{X} < \frac{1}{2}\,l, \qquad C = C_1$$

$$t > 0, \qquad \mathcal{X} = \pm\frac{1}{2}\,l, \qquad C = C_0$$

Where $l$ is the thickness of the dialysis membrane (cellular membrane, thickness = 0.02 μm). Under the above-specified boundary conditions, Fick's second law in the form of a trigonometric series is Equation 6.2:

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(n+1)^2} exp\left\{ - \frac{(2n+1)^2 D\pi^2}{l^2} t \right\}$$

Equation 6.2

Where $M_t$ and $M_\infty$ are the amount of drug released at time $t$ and infinite time, respectively, and π is the ratio of a circle's circumference to its diameter (π = 3.1415). By taking the first term in the summation (Σ) series and performing a logarithmic transformation of Equation 6.2, Equation 6.3 is obtained. From this the diffusion coefficient can be determined [210]. This is achieved by plotting ln(1-($M_t/M_\infty$)) versus $t$, then the slope, $-D\pi^2/l^2$, of the linear plot can be used to determine the value of $D$.

$$ln\left(1 - \frac{M_t}{M_\infty}\right) = ln\ \frac{8}{\pi^2} - \frac{D\pi^2}{l^2}$$

Equation 6.3

## 6.4 Fractional amount of CPT release

The CPT/β-CD and CPT/β-CD-g-Alg inclusion complexes were prepared by a co-precipitation method (see Chapter 2, Sections 2.10.1 and 2.10.2.2, respectively, for the full synthesis method). *In vitro* release profiles of free CPT and CPT from CPT/β-CD and CPT/β-CD-g-Alg inclusion complexes were studied by a dialysis method in Tris buffer solution pH ~ 7.4 at 37 °C (see Chapter 2, Section 2.12 for the full details). The same type of dialysis membrane (cellular membrane) was used in all the release experiments.

### 6.4.1 CPT release measured by UV-Vis spectroscopy

UV-visible spectrophotometry was used to determine the concentration of CPT instead of the fluorescence spectrophotometry. This change was made as it was observed that the absorption spectrum red-shifted at a smaller range, ~5 nm, than the emission spectrum, ~20 nm, when the pH was changed [84, 167]. The concentration of CPT in the Tris buffer solution was calculated from the CPT calibration curve, which was developed previously from known concentrations of CPT (Refer to Appendices Figure 3). From this data the fractional amount ($M_t/M_\infty$) of CPT was calculated.

Figure 6.1, 6.2 and 6.3 shows the change in the UV-vis spectra of CPT released at pH~7.4 at 37 °C from a dialysis membrane for free CPT, and the CPT/β-CD and CPT/β-CD-g-Alg inclusion complexes, respectively. These figures show that the absorbance intensity of the Tris buffer solution at 365 nm for the CPT increases with increasing time. There is clearly a significant difference in the change in the absorbance intensity with time for all of the three figures. In particular, Figure 6.1 shows a rapid change in absorbance intensity with increasing time indicating the fast increase of free CPT through the dialysis membrane.

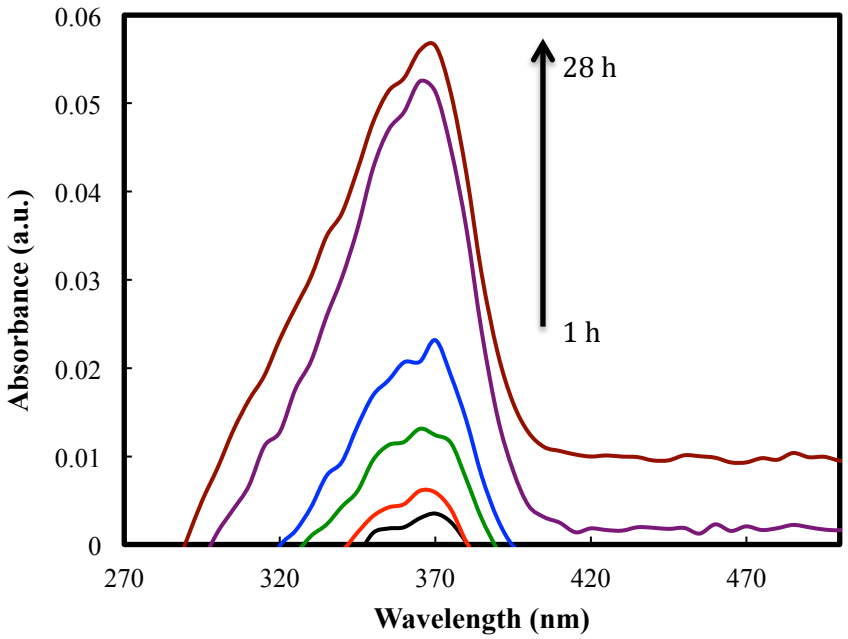

Figure 6-1 UV-Vis spectra of Tris buffer solution at pH ~ 7.4 and at 37 °C in the release experiment of free CPT from a dialysis membrane.

Figure 6.2 shows the change in the absorbance intensity of the release of CPT from the CPT/β-CD inclusion complex with increasing time. As can be seen from Figure 6.2 the change in the absorbance intensity in the beginning is fast then it becomes slow. This will be explained later in this Chapter.

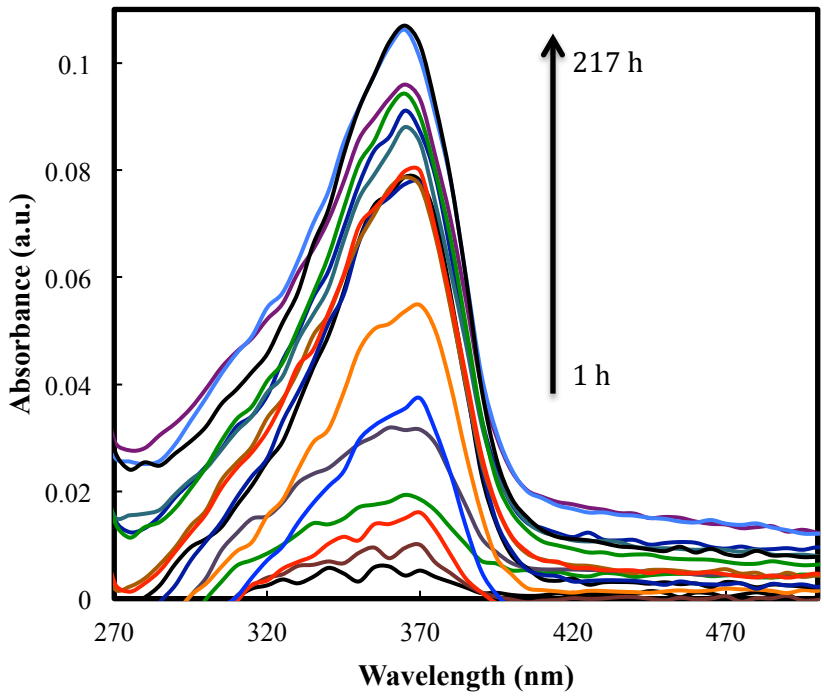


Figure 6-2 UV-Vis spectra of Tris buffer solution at pH ~ 7.4 and at 37 °C in the release experiment of CPT from the CPT/β-CD inclusion complex.

The change in the absorbance intensity of the release of CPT from the CPT/β-CD-g-Alg inclusion complex with increasing time is presented in Figure 6.3. It can be seen from Figure 6.3 the change in the absorbance intensity gradually increases with increasing time.

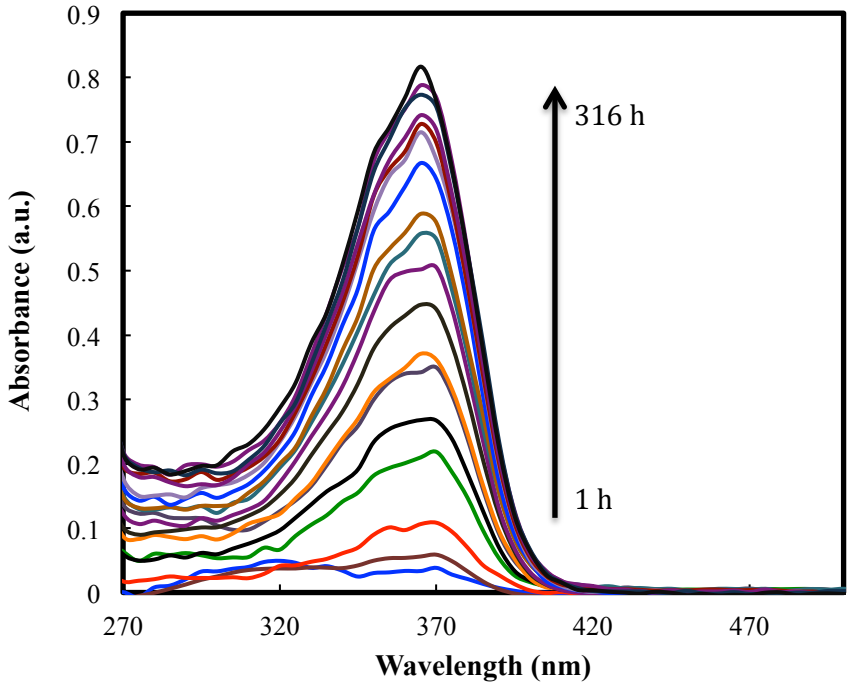


Figure 6-3 UV-Vis spectra of Tris buffer solution at pH ~ 7.4 and at 37 °C in the release experiment of CPT from CPT/β-CD-g-Alg inclusion complex.

This UV-vis data was then used to calculate the fractional release.

### 6.4.2 Fractional amount of free CPT release and CPT release from CPT/β-CD and CPT/β-CD-g-Alg inclusion complexes

Figure 6.4 (red circles) shows the fractional release of CPT through the dialysis membranes. As can be seen from Figure 6.4 the free CPT is nearly completely released within 24 h. A similar result has been observed previously in literature [104, 116], where 24 h was enough time to completely release free CPT from the dialysis membranes.

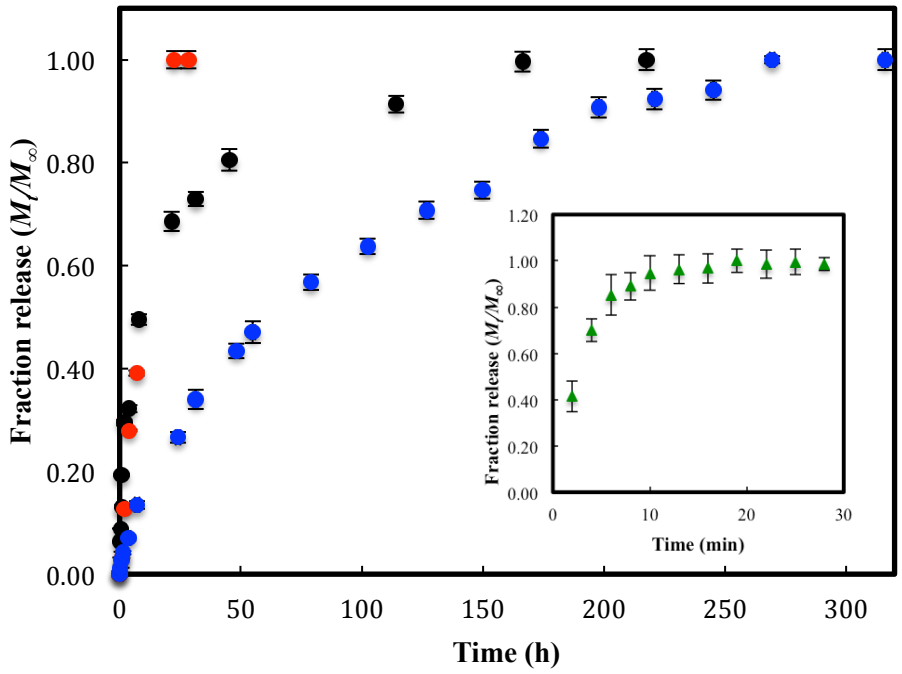


Figure 6-4 Fraction release, $M_t/M_\infty$ of free CPT (red circles), CPT from CPT/β-CD inclusion complex (black circles) and CPT from CPT/β-CD-g-Alg inclusion complex (blue circles). Insert: Fraction release, $M_t/M_\infty$ of CPT from Ca-$Alg_2$ hydrogel discs at pH ~ 7.4.

Figure 6.4 also shows the fraction release of CPT from the CPT/β-CD inclusion complex (Figure 6.4, black circles) and CPT from the CPT/β-CD-g-Alg inclusion complex (Figure 6.4, blue circles). As shown in Figure 6.4 an initial burst release was observed for CPT from the CPT/β-CD inclusion complex (Figure 6.4 black circles) in the first 7~8 hours. This was then followed by a delayed release, which reached a plateau after 9 days. Compared to the free CPT the presence of β-CD significantly changes the release time as well as the release profile. The slower release of CPT from the CPT/β-CD inclusion complex is attributed to host-guest complex formation between the CPT molecules and the CPT/β-CD. The β-CD plays a role here by slowing the CPT release as β-CD's interior cavity has a pronounced hydrophobic character which allows β-CD to host hydrophobic CPT molecules efficiently in aqueous solutions for long periods of time (see Chapter 1, Figure 1.15) [108]. This is achieved through hydrophobic-hydrophobic interactions (van der Waals interactions) between the hydrophobic moiety of the CPT and the β-CD cavity, and the hydrogen bonding between the polar functional groups in CPT and the hydroxyl groups in β-CD

[211]. This is observed as a slower release of CPT from the CPT/β-CD inclusion complex (Figure 6.4 black circles).

Interestingly, the release of CPT from the CPT/β-CD-g-Alg inclusion complex did not appear to show any significant burst release and the CPT increased gradually until reaching equilibrium after 13 days. The slower release of CPT from the CPT/β-CD-g-Alg inclusion complex may be attributed to both the mucoadhesive property of Na-Alg and the host-guest complex formation between the CPT molecules and the CPT/β-CD-g-Alg. Na-Alg not only increases the aqueous solubility of β-CD and CPT/β-CD inclusion complexes, but also enhances the formation of the CPT/β-CD inclusion complex [212]. Loftsson et al. [213] studied the change in the solubility of β-CD and different drug/β-CD complexes in the presence of hydroxypropylmethylcellulose (HPMC) or polyvinylpyrrolidone (PVP) or carboxymethylcellulose (CMC). They observed that the presence of any of the polymers increased the solubility of β-CD and drug/ β-CD complexes [213]. They also found that the incorporation of the drug into β-CD enhanced with the polymers [213]. Cango et al. [214] studied the potential of β-cyclodextrin-dextran polymer (β-CD-dextrin) for drug delivery. They investigated the influence of the dextran backbones on the solubilisation efficiency of hydrocortisone (HC) (hydrophobic drug), stability of the β-CD/HC complex and the release profile. They found that the presence of dextran backbone increased the solubility of β-CD/HC complex. They observed the lower release of HC through the dialysis membrane when it is associated with β-CD-dextrin in comparison to the HC suspension [214].

When comparing the release of CPT molecules from the CPT/β-CD-g-Alg inclusion complex (Figure 6.4, blue circles) and Ca-$Alg_2$ hydrogel discs (Figure 6.4, insert) (see also Chapter 4, Section 4.5.1.2, Figure 4.11) it is clear that the release of CPT molecules from Ca-$Alg_2$ hydrogel discs was very fast, reaching equilibrium after 16 min (Refer to Chapter 4, Section 4.5.1.2 for full details). In comparison, the release of CPT molecules from the CPT/β-CD-g-Alg inclusion complex was very slow, reaching equilibrium after 13 days. It is clear that the modification of Na-Alg by grafting with β-CD have proven to be useful for the slow release of the hydrophobic CPT molecules.

The following section describes how the fractional release was used to calculate the release diffusion coefficients for the release of free CPT and the release of CPT from the CPT/β-CD and CPT/β-CD-g-Alg inclusion complexes.

It observed that the slow release of CPT from CPT/β-CD inclusion complex is attributed to the host-guest complex formation between CPT and β-CD. In comparison, the release of CPT from CPT/β-CD-g-Alg inclusion complex was very slow due to the host-guest complex formation between CPT and β-CD as well as the mucoadhesive property of Na-Alg, which enhances the formation of the CPT/β-CD inclusion complex.

## 6.5 Diffusion coefficient of CPT release

### 6.5.1 Fitting Fick's second law

Given that the release of free CPT shows only burst release (fast release) then only one fit is required. However, for the release of CPT from the CPT/β-CD inclusion complex where the first stage was burst release, occurring within the first 8 h, and the second stage was the slow release of CPT molecules from the inclusion complex it is necessary and reasonable to therefore fit the two stages separately on two different curves [209]. In this case, Figure 6.5 (a and b) were plotted from the data in Figure 6.4, by applying Equation 6.3. Figure 6.5(a) shows the semi-logarithmic plots of the data in Figure 6.4 for the first stage release for free CPT molecules (Figure 6.5(a) red circles), and for CPT from the CPT/β-CD inclusion complex (Figure 6.5(a) black circles) as a function of time. It can be seen that the coefficient constants, $R^2$ are reasonably high at 0.9790 and 0.9733 for free CPT release and CPT released from the CPT/β-CD inclusion complex, respectively, suggesting that the Fick's second law equation fits the release data well.

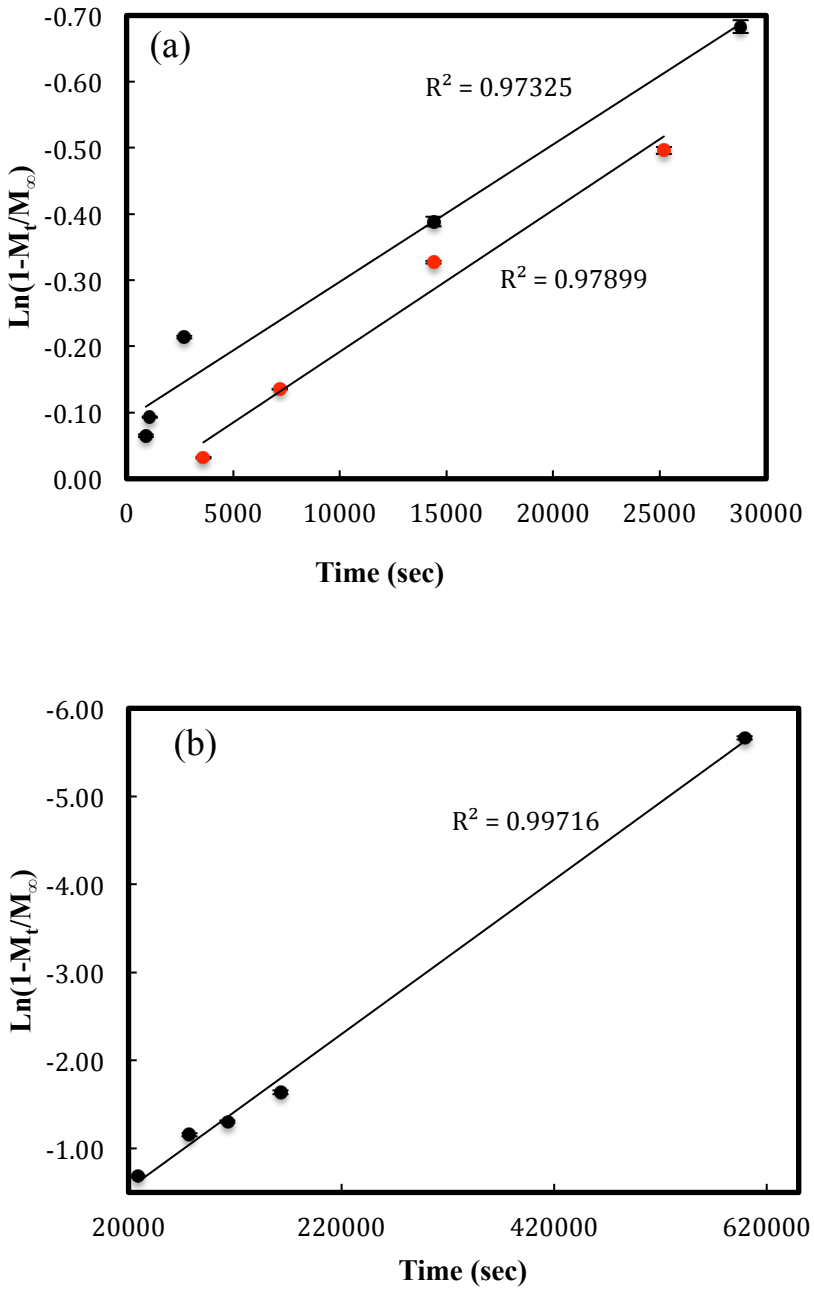


Figure 6-5 Semi-logarithmic plots of the data in Figure 6.4 as a function of time for free CPT (red circles) and CPT from CPT/β-CD inclusion complex (black circles), (a) is a first stage (fast release) and (b) is a second stage (slow release).

The semi-logarithmic plot of the data in Figure 6.4 for the second stage release for CPT from the CPT/β-CD inclusion complex is shown in Figure 6.5(b). The coefficient constants, $R^2$ for this also implies a good fit to Fick's second law at 0.9972.

The release of CPT from CPT/β-CD-g-Alg inclusion complex shows only the slow release because there is no burst release; subsequently only one fit is required. Figure 6.6 represents the semi-logarithmic plot of the data in Figure 6.4 for the release of CPT from the CPT/β-CD-g-Alg inclusion complex. A coefficient constant, $R^2$, of 0.9852 implies a good fit.

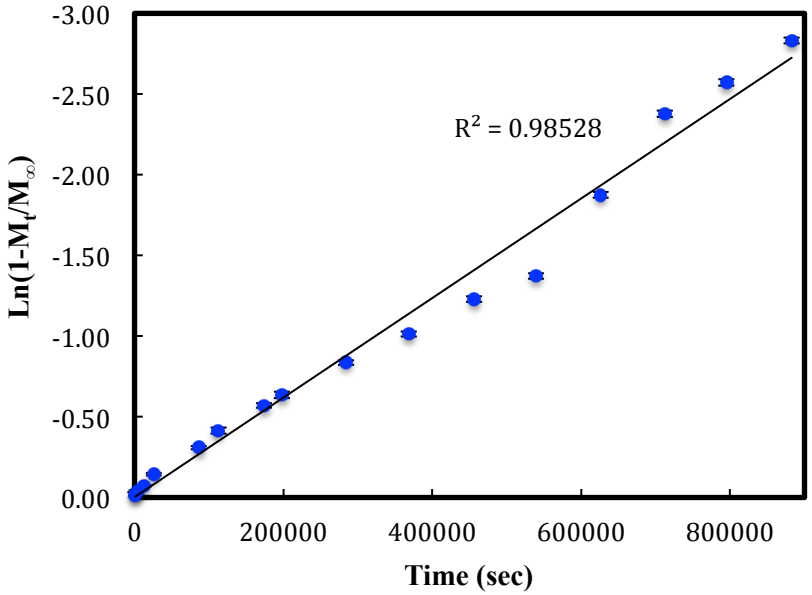


Figure 6-6 Semi-logarithmic plot of the data in Figure 6.4 as a function of time for CPT from a CPT/β-CD-g-Alg inclusion complex.

### 6.5.2 Release diffusion coefficients

The release diffusion coefficients have been designated as the following: $D_1$, is the release diffusion coefficient for the first stage (fast release) and $D_2$, is the release diffusion coefficient for the second stage (slow release). Both $D_1$ and $D_2$ were obtained from Figure 6.5 and 6.6 and are summarised in Table 6.1. As can be seen from Table 6.1, the $D_1$ values for the release of free CPT and the release of CPT from the CPT/β-CD inclusion complex are the same at $8.113 \times 10^{-10}$ $mm^2$ $s^{-1}$, indicating that some of CPT molecules did not form an inclusion complex with β-CD. Here it is theorised that when the CPT/β-CD inclusion complex was exposed to the Tris buffer solution the CPT molecules, which were not included into the β-CD cavity, were initially released followed by the CPT molecules, which were included in the β-CD cavity as confirmed by ATR-FTIR and $^1$H-NMR results (Refer to Chapter 5, Section's

5.5.1.1 and 5.5.1.2 for full details). A similar result was observed by De Jesus et al. [215]. They used diffusion ordered spectroscopy to determined the diffusion coefficient of pure riboflavin (RF) (a hydrophobic drug) and a RF/β-CD complex [215]. They found that the diffusion coefficient of pure RF was $3.23\times 10^{-10}$ $mm^2$ $s^{-1}$ while RF from RF/β-CD complex was similar at $3.13\times 10^{-10}$ $mm^2$ $s^{-1}$. They attributed this result to the interaction between RF and β-CD showing non-inclusion of RF in the complex [215].

Table 6-1 The release diffusion coefficients of free CPT and CPT from the CPT/β-CD and CPT/β-CD-g-Alg inclusion complexes in Tris buffer.

| **CPT in** | **$D_1 \times 10^{-10}$ ($mm^2$ $s^{-1}$)** | **$D_2 \times 10^{-10}$ ($mm^2$ $s^{-1}$)** |
|---|---|---|
| **Tris buffer only** | 8.113 ± 0.945 | - |
| **CPT/β-CD** | 8.113 | 3.651 ± 1.01 |
| **CPT/β-CD-g-Alg** | - | 1.217 |

The $D_2$ for the release of CPT from the CPT/β-CD and the CPT/β-CD-g-Alg inclusion complexes are shown in Table 6.1. The slower release diffusion coefficients observed for both inclusion complexes can be explained by the release of included CPT from the β-CD cavities. By comparison the $D_2$ for the release of CPT from the CPT/β-CD-g-Alg inclusion complex (at $1.217 \times 10^{-10}$ $mm^2$ $s^{-1}$) is slower than the $D_2$ for the release of CPT from the CPT/β-CD (at $3.651 \times 10^{-10}$ $mm^2$ $s^{-1}$), indicating that the Na-Alg increased the solubility of CPT/β-CD and thus enhanced CPT/β-CD formation. This result suggests that β-CD-g-Alg, as a new drug carrier, holds great potential as a prolonged delivery system for controlled release of poorly water-soluble drugs.

Of most interest here is that the CPT release from the CPT/β-CD-g-Alg inclusion complex has no burst release. This is an advantage as burst release is deemed a major problem in the development of controlled drug release systems [216] because drugs are delivered at too high doses/concentrations which in turn can result in increased toxicity [216].

Most of the published work related to burst release has focused on ways to minimise or prevent it from occurring in controlled release system. Thote et al. [217] produced poly(lactide-co-glycolide) (PLGA) microparticles containing nanoparticles of the hydrophilic drug dexamethasone phosphate. They found that these particles provided sustained release of dexamethasone phosphate without an initial burst release. Hasan et al. [216] encapsulated poly-ε-caprolactone (PCL) nanoparticles into polymeric microparticles of ethylcellulose and Eudragit RS using a water in oil in water (W/O/W) emulsion for the release of ibuprofen and triptorelin acetate. They found that the burst release significantly decreased with composite microparticles, and they explained this by the slower diffusion of the drugs through the double polymeric walled system [216]. Here CPT/β-CD inclusion complexes showed prolonged release of CPT with initial burst release due to the free CPT molecules, which were not included into β-CD cavity. However, in the formation of CPT/β-CD-g-Alg inclusion complexes all CPT was included into the β-CD cavity. That means there are no free CPT molecules in the CPT/β-CD-g-Alg inclusion complex and as a result the burst release was not observed in the release of CPT from CPT/β-CD-g-Alg inclusion complex.

## 6.6 Concluding remarks

This chapter investigated the release profiles of free CPT molecules and CPT molecules from CPT/β-CD and CPT/β-CD-g-Alg inclusion complexes. The free CPT in Tris buffer solution only was completely released in 24 h. The release of CPT from CPT/β-CD inclusion complex showed two stages. The first stage (initial burst release) is related to the release of CPT molecules that were not included in the β-CD. It was found that the initial burst release was in the first 7-8 hours. However, the second stage (slow release) was related to the release of CPT from the β-CD cavity. It was found that the release of CPT from the CPT/β-CD inclusion complex increased gradually until an equilibrium was reached after 9 days.

In contrast, the release of CPT from the CPT/β-CD-g-Alg inclusion complex did not show the initial burst release because all of the CPT molecules were either bound to the Na-Alg or included into the β-CD cavity. It was found that the release of CPT from the CPT/β-CD-g-Alg inclusion complex showed a constant slow release until an equilibrium was reached after 13 days. The slower release of CPT from the CPT/β-CD-g-Alg inclusion complex can be attributed to the release of CPT molecules from the β-CD cavities and from the Na-Alg mucoadhesive matrix. Additionally, the release diffusion coefficients were calculated from Fick's second law. It was found that the $D_1$ values for the release of free CPT and the release of CPT from the CPT/β-CD inclusion complex were the same at $8.113 \times 10^{-10}$ $mm^2$ $s^{-1}$, indicating that some of the CPT molecules did not form an inclusion complex with β-CD. The $D_2$ for the release of CPT from the CPT/β-CD was ($3.651 \times 10^{-10}$ $mm^2$ $s^{-1}$), indicating the increased solubility of the CPT. It was found that the $D_2$ values for the release of CPT from the CPT/β-CD-g-Alg inclusion complex showed slower diffusion at $1.217\times 10^{-10}$ mm $s^{-1}$ than that of the CPT/β-CD at $3.651 \times 10^{-10}$ mm $s^{-1}$, indicating a slow release caused by β-CD-g-Alg. This can be explained by the fact that Na-Alg increases the solubility of the CPT/β-CD inclusion complex and hence enhances the formation of CPT/β-C.